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**Name of Organization:** University of MI, Ctr. for Great Lakes & Aquatic Sciences

**Type of Organization:** College or University

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**Project Title:** Characterization of Tumors in Great Lakes Zooplankton

**Project Category:** Emerging Issues

**Rank by Organization (if applicable):** 0

**Total Funding Requested (\$):** 171,314 **Project Duration:** 2 Years

**Abstract:**

In 1998 we found large tumorous abnormalities on several species of Great Lakes zooplankton and published the first paper documenting them in North America (Omair et al. 1999:CJFAS). Samples from our perch study conducted at 3-m and 6-m depths revealed 11 different affected species, one with 70% prevalence rates of tumors. Our work has demonstrated that these tumors consist of degenerated, necrotic tissue derived from living tissue(s) that has been extruded from within the organism through the body wall. Tumors have been found in nauplii, copepodites, adults of both sexes, and in exotic species. We believe that these lesions, some of which are 20% of the size of the organism, are fatal by impeding feeding behavior or movement, making the organism susceptible to starvation and predation. This appears to be a recent phenomenon in North America and may be global. Cause of the tumors is unknown, but implications for the health of the Great Lakes fauna, especially zooplankton, which are already stressed from zebra mussels and declining P levels, are cause for grave concern. Zooplankton are vital links in converting phytoplankton into food for higher organisms in the food chain. Thus, they are an indispensable source of food for obligate planktivores. Since we first found these tumors, we have received help in characterizing them from the faculty of the Univ. MI Medical Center: T. Beals and B. Naylor, pathologists, and J. Quddus, immunologist. To continue the work, we propose to: 1.) conduct more extensive anatomic and pathologic analysis of fresh specimens, 2.) provide prevalence, distribution, and mortality data for zooplankton with tumors by examination of archived zooplankton samples and performance of aquarium experiments, 3.) perform Mass-Spec analyses on algae and zooplankton collected from Lake Michigan and a control area, and 4.) expose zooplankton to various factors (Lake Michigan water, algae, zebra mussels) in an attempt to induce tumors.

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**Geographic Areas Affected by the Project**

**States:**

<input type="checkbox"/> Illinois	<input type="checkbox"/> New York
<input type="checkbox"/> Indiana	<input type="checkbox"/> Pennsylvania
<input checked="" type="checkbox"/> Michigan	<input type="checkbox"/> Wisconsin
<input type="checkbox"/> Minnesota	<input type="checkbox"/> Ohio

**Lakes:**

<input type="checkbox"/> Superior	<input type="checkbox"/> Erie
<input type="checkbox"/> Huron	<input type="checkbox"/> Ontario
<input checked="" type="checkbox"/> Michigan	<input checked="" type="checkbox"/> All Lakes

**Geographic Initiatives:**

☐ Greater Chicago   ☐ NE Ohio   ☐ NW Indiana   ☐ SE Michigan   ☐ Lake St. Clair

**Primary Affected Area of Concern:** Muskegon Lake, MI

**Other Affected Areas of Concern:** I

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***For Habitat Projects Only:***

**Primary Affected Biodiversity Investment Area:**

**Other Affected Biodiversity Investment Areas:**

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**Problem Statement:**

Zooplankton in Lake Michigan, of many different species and stages of development, have been found to bear tumors. After this was documented, reports of their presence came in from many other areas throughout the Great Lakes, and also from a Michigan inland lake and a tributary to Lake Erie. Over 41% of the species of common Great Lakes zooplankton have been found to bear tumors. We believe this is a recent phenomenon, since, prior to our documentation, it had not been reported by our taxonomist (M. Omair) despite his 30 yr of experience in identifying zooplankton nor by any other groups examining Great Lakes zooplankton. Reports of grossly similar lesions ("cysts") from Europe pre-dated our findings leading us to believe that such tumor development may have occurred at such low levels that it went undocumented. It is clear that the dramatic tumor prevalence rates we are currently experiencing are unprecedented and represent either a new agent inducing this response, or some low-level process that formerly induced the tumors at a low rate which has now accelerated.

The cause(s) of the tumors remains speculative, such as riverborne or airborne toxic substances or viruses or other pathogens, which, in turn, may be associated with the widespread presence of zebra mussels. Zooplankton are already stressed because of declining food supplies caused by the phosphorus decline and competition from zebra mussels. This may have changed food quality and contaminant cycling relationships. Since all zooplankton with tumors that we examined were dead, whereas those in the same sample without tumors were alive, we are led to believe that these tumors are lethal lesions because they impair movement and feeding of affected zooplankton, rendering them vulnerable to predation and starvation.

After finding these anomalies, we gained assistance from three UM medical doctors, who have been characterizing the tumors histologically and bacteriologically. A considerable amount of our histological tumor knowledge is due to their efforts. Their work has shown that the tumors appear to be derived from viable tissue within the organism and were then exuded through cracks in the exoskeleton. No parasites or fungi have been observed in relation to the tumors, but on some specimens there was a heavy coating of bacilli on the outside of the tumors.

Since none of the work we have done to date has been funded, we have not been able to provide the amount of time and obtain the kind of fresh samples or ensure the preservation of samples that is necessary for optimal results. Hence, for the continuation of this effort, we propose that additional fresh samples be provided to the pathologists from our Muskegon site on Lake Michigan (ongoing yellow perch study) and from other sites as available. Jude has some support (ca. 15K - match) for collecting zooplankton samples as part of a Great Lakes Fisheries Trust Grant. Nearshore samples from this site have the highest recorded prevalence and diversity of affected species of any samples observed so far. Fresh samples would be provided from this site to Drs. Naylor, Beals, and Quddus, who will then have optimal samples with which to characterize both the specimens that do not have tumors (to learn more about what "normal" tissue looks like) and a range of zooplankton species that do have tumors during different seasons of the year. In addition, we need more information on what species are affected and current and historical prevalences of tumors on Great Lakes zooplankton. Samples to accomplish the latter goals are currently available, having been collected recently (1998-1999), and will be collected during

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2000-2002 as part of an ongoing project. To address what induces these tumors, some experiments need to be done to eliminate some of the hypotheses that have currently been advanced to account for their presence. These include toxic substances or other compounds that might be endocrine disrupters or damage chitin, and pathogens or viruses associated with zebra mussels and algal extracts. An experimental approach using aquaria might provide ready answers to some of these questions.

#### **Proposed Work Outcome:**

We propose four major tasks for this study: 1.) More extensive anatomic and pathologic investigation of the tissues of normal zooplankton and those with tumors by use of light microscopy and scanning and transmission electron microscopy for histological analysis, and documentation and classification of any bacteria present. 2.) Provide prevalence, distribution, and mortality data for zooplankton with tumors. Here there are four components. First, we will examine some of the archived samples we have from the 1970-80s from SE Lake Michigan to determine if any zooplankton had tumors. Second, we propose to count the number of zooplankton with tumors in the zooplankton samples we collected in Lake Michigan at 3-m and 6-m depths at Muskegon, MI to quantify the seasonal prevalence of tumors. We have triplicate samples at each depth that were collected roughly weekly from late May-August and bimonthly samples from September-October, 1998-99. Third, we will continue to measure the prevalence, seasonal distribution, and progress of tumor development in zooplankton during ongoing 2000-2002 yellow perch studies. Fourth, we will perform some simple aquarium experiments lakeside taking freshly collected zooplankton and determining if and how fast those with tumors die. 3.) Algae and zooplankton collected from two sites - Lake Michigan near Muskegon (presence of zebra mussels) and a control site (large oligotrophic inland lake without zebra mussels)- will be screened for toxic and unusual compounds three times, spring, summer, and fall. Algae will be collected with a 10-micron plankton net (pre-screened with a 156-micron net to remove most zooplankton) and zooplankton will be collected with a 156- micron net. A mass spectrometer will be used at Grand Valley State University for these analyses (directed by Richard Rediske). 4.) Laboratory experiments will be planned to try to induce tumors in zooplankton collected from a zebra mussel-free lake. Several treatments are planned including: using

filtered Muskegon intake water from Lake Michigan and algal food from a pristine lake (to isolate water-borne compounds that may induce tumors), using unfiltered Muskegon intake water (would include algae, zebra mussels, and associated compounds), using algae collected from Lake Michigan as food, adding live zebra mussels in with pristine zooplankton, and running the Lake Michigan water through a carbon filter first before use, and a control.

The outcomes of this research will provide critical information on several fronts. First, it may be that some of the specimens analyzed to date were deteriorating when histological analyses were done. Fresh specimens or appropriately preserved ones will be provided to the pathologists and bacteriologists to ensure their analyses are done on specimens in good condition. This will eliminate some of the speculation as to why the contents of some of the tumors often appear to be just necrotic cellular debris, and whether the presence of bacteria on the tumor was an antemortem or postmortem phenomenon. It will also allow pathologists to analyze a series of tumors as the season progresses, characterize newly formed tumors, and to do more work on "normal" tissue. Second, we need to know if these tumors existed prior to the 1990s and if they did, their prevalence rates. We have archived zooplankton samples from the 1970s and 80s, which can be analyzed to provide this information. Third, we need some information on mortality of zooplankton with tumors. This grant will allow us to bring in fresh samples to the onshore lab at Muskegon and determine how long zooplankton with tumors live in this situation. Fourth, we will have prevalence data on tumorous zooplankton from a nearshore area, where our highest rates have been recorded, and where it would be expected that zebra mussels would have an effect and where input of river-borne anthropogenic substances would be maximal. In addition, our nearshore data from 1998-2002 will complement other datasets that might be available from NOAA and EPA which focus their efforts far offshore. Lastly, the aquaria experiments will provide some information on whether tumors can be induced from Lake Michigan water or by zebra mussels and algae from Lake Michigan. We hope these data will provide critical information on the causes of tumors on zooplankton, to help form strategies to combat their development, assessing whether there might be other members of the food chain at risk, and finally, whether the health of humans is put at risk.

**Project Milestones:**

**Dates:**

Project Start	01/2001
Collection of zooplankton field samples	05/2001
Histological and pathological analyses	05/2001
Counting 1998-1999 zooplankton samples	01/2001
Mortality experiments	05/2001
Measure toxic substances via MS	05/2001
Induction experiments	05/2001
Project End	12/2002

☐ Project Addresses Environmental Justice

**If So, Description of How:**

☐ Project Addresses Education/Outreach

**If So, Description of How:**

In so much as this is an important topic and we have already had considerable contact with the media, we intend to maintain good relations with media and report our results when they are defensible. We will also present our work at conferences, especially the IAGLR conference, where our previous work was reported.

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**Project Budget:**

	<b>Federal Share Requested (\$)</b>	<b>Applicant's Share (\$)</b>
<b>Personnel:</b>	80,891	4,729
<b>Fringe:</b>	17,562	1,324
<b>Travel:</b>	6,000	0
<b>Equipment:</b>	0	0
<b>Supplies:</b>	9,000	0
<b>Contracts:</b>	0	0
<b>Construction:</b>	0	0
<b>Other:</b>	0	0
<b>Total Direct Costs:</b>	113,453	6,053
<b>Indirect Costs:</b>	57,861	3,087
<b>Total:</b>	171,314	9,140
<b>Projected Income:</b>	0	0

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**Funding by Other Organizations (Names, Amounts, Description of Commitments):**

We have a Great Lakes Fishery Trust grant to study yellow perch recruitment in Lake Michigan at Muskegon for 2000-2002, which will provide the samples of zooplankton to be evaluated for tumors. Match will be provided by the University of Michigan (CLGAS) and the Office of the Vice President for Research.

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**Description of Collaboration/Community Based Support:**

We have not yet developed viable collaboration and community support, but we expect to gain it from the positive responses we have received from the various news media, which have contacted us and discussed what was being done about this problem. We will contact various groups including those working with the White Lake and Muskegon Lake AOCs. We have collaborated with the Great Lakes Fishery Commission sponsored Yellow Perch Task force, which collects zooplankton as part of the recruitment analyses; some members have sent us samples with afflicted zooplankton. We first discovered the tumors in 1995 in a specimen submitted by GLERL. We have also been in contact with EPA's surveillance group, which also discovered tumors on zooplankton in some of their samples from Lakes Erie and Michigan. Our data will not duplicate any ongoing studies by GLERL or EPA, since our sites are much closer to shore, where they seldom or ever sample. Thus our data will complement their offshore data.